Supplemental Table 1. Analytical reactivity and cross-reactivity analyses.

Organism				**Source**	**Test concentration**	**Total positives by Singulex Clarity C. diff toxins A/B assay (n, number of replicates tested)**
***C. difficile* strains**						
ATCC Strain	**Toxinotype**	**Ribotype**	***tcdA*/*tcdB* status**			
630	0	012	+/+	ATCC BAA-1382	3.7 x 10^8 cells/mL	3/3
N/A	IIIb	027	+/+	ATCC BAA-1805	6.5 x 10^8 cells/mL	3/3
VPI 10463	0	087	+/+	ATCC 43255	6.9 x 10^8 cells/mL	3/3
W1194	0	005	+/+	ATCC 43594	6.3 x 10^8 cells/mL	3/3
545	0	012	+/+	ATCC 43596	1.9 x 10^8 cells/mL	3/3
2022	0	001	+/+	ATCC 43599	1.3 x 10^8 cells/mL	3/3
2149	0	014	+/+	ATCC 43600	1.1 x 10^8 cells/mL	3/3
870	0	001	+/+	ATCC 17857	1.7 x 10^8 cells/mL	3/3
1253	0	054	-/+	ATCC 17858	4.1 x 10^8 cells/mL	3/3
14797-2	0	005	+/+	ATCC 700792	2.1 x 10^8 cells/mL	3/3
Not ATCC strain	0	220	+/+	ATCC BAA-1806	1.2 x 10^8 cells/mL	3/3
Not ATCC strain	0	020	+/+	ATCC BAA-1808	7.5 x 10^8 cells/mL	3/3
Not ATCC strain	0	057	+/+	ATCC BAA-1811	8.5 x 10^8 cells/mL	3/3
Not ATCC strain	0	002	+/+	ATCC BAA 1813	8.6 x 10^8 cells/mL	3/3
Not ATCC strain	0	076	+/+	ATCC BAA-1815	7.7 x 10^7 cells/mL	3/3
4206	0	207	+/+	ATCC BAA-1872	4.2 x 10^8 cells/mL	3/3
LBM 0801058	XXII	251	+/+	ATCC BAA-2155	5.8 x 10^8 cells/mL	3/3
LBM 0801040	0	118	+/+	ATCC BAA-2156	5.0 x 10^8 cells/mL	3/3
N/A	0	014	+/+	ATCC 43597	5.9 x 10^8 cells/mL	2/3
BDMS 18 AN	0	001	+/+	ATCC 51695	2.2 x 10^8 cells/mL	3/3
3232	N/A	010	-/-	ATCC BAA-1801	7.9 x 10^8 cells/mL	0/3
Not ATCC strain	IIIc	027	+/+	ATCC BAA-1803	7.8 x 10^8 cells/mL	3/3
Not ATCC strain	0	053	+/+	ATCC BAA-1804	7.8 x 10^8 cells/mL	3/3
Not ATCC strain	XII	024	+/+	ATCC BAA-1812	8.1 x 10^8 cells/mL	3/3
5325	V	078	+/+	ATCC BAA-1875	7.2 x 10^8 cells/mL	3/3
90556-M6S	0	001	+/+	ATCC 9689	6.8 x 10^8 cells/mL	2/2
1470	VIII	017	-/+	ATCC 43598	8.4 x 10^8 cells/mL	2/2
Not ATCC strain	XXII	251	+/-	ATCC BAA-1814	8.8 x 10^8 cells/mL	3/3
4118	IIIb	027	+/+	ATCC BAA-1870	7.7 x 10^8 cells/mL	3/3

1351	N/A	060	-/-	ATCC 43593	6.1 x 10^8 cells/mL	0/3
7322	N/A	031	-/-	ATCC 43601	1.3 x 10^7 cells/mL	0/3
4111	0	001	+/+	ATCC BAA-1871	2.4 x 10^8 cells/mL	3/3
5283	0	053	+/+	ATCC BAA-1873	1.4 x 10^8 cells/mL	3/3
4205	0	002	+/+	ATCC BAA-1874	1.3 x 10^8 cells/mL	3/3
CCUG 20309	N/A	N/A	N/A	CCUG 26891	1.6 x 10^8 cells/mL	3/3
CCUG 46469	N/A	N/A	N/A	CCUG 26893	2.6 x 10^8 cells/mL	3/3
CCUG 64977	N/A	N/A	N/A	CCUG 26894	3.0 x 10^8 cells/mL	3/3
Aerobic bacteria						
Abiotrophia defectiva				ATCC 49176	2.5 x 10^8 cells/mL	0/3
Acinetobacter baumannii				ATCC 19606	5.5 x 10^8 cells/mL	0/3
Aeromonas hydrophila				ATCC 7966	9.4 x 10^8 cells/mL	0/3
Alcaligenes faecalis				ATCC 15554	7.0 x 10^8 cells/mL	0/3
Bacillus cereus				ATCC 13472	7.4 x 10^8 cells/mL	0/3
Citrobacter freundii				ATCC 8090	8.7 x 10^8 cells/mL	0/3
Edwardsiella tarda				ATCC 15947	8.8 x 10^8 cells/mL	0/3
Enterobacter aerogenes				ATCC 13048	5.3 x 10^8 cells/mL	0/3
Enterobacter cloacae				ATCC 13047	8.7 x 10^8 cells/mL	0/3
Enterococcus faecalis vanB				ATCC 51299	8.0 x 10^8 cells/mL	0/3
Escherichia coli				ATCC 23511	7.5 x 10^8 cells/mL	0/3
Escherichia coli O157:H7				ATCC 700927	1.1 x 10^9 cells/mL	0/3
Plesiomonas shigelloides				CCUG 26892	4.2 x 10^8 cells/mL	0/3
Proteus mirabilis				ATCC 25933	9.8 x 10^8 cells/mL	0/3
Providencia alcalifaciens				ATCC 9886	7.7 x 10^8 cells/mL	0/3
Pseudomonas aeruginosa				ATCC 10145	1.0 x 10^7 cells/mL	0/3
Salmonella arizonae				ATCC 13314	8.5 x 10^8 cells/mL	0/3
Salmonella enterica				ATCC 7001	6.5 x 10^8 cells/mL	0/3
Salmonella typhimirium				ATCC 29630	1 x 10^7 cells/mL	0/3
Serratia liquefaciens				ATCC 27592	8.7 x 10^8 cells/mL	0/3
Serratia marcescens				ATCC 13880	8.2 x 10^8 cells/mL	0/3
Shigella boydii				ATCC 9207	8.4 x 10^8 cells/mL	0/3
Shigella dysenteriae				ATCC 11835	4.1 x 10^8 cells/mL	0/3
Shigella flexneri				ATCC 12022	1.0 x 10^7 cells/mL	0/3
Shigella sonnei				CCUG 26896	1.9 x 10^8 cells/mL	0/3
Staphylococcus aureus				ATCC 43300	1.0 x 10^9 cells/mL	0/3
Staphylococcus epidermidis				ATCC 14990	6.8 x 10^8 cells/mL	0/3
Streptococcus agalactiae				ATCC 12973	1.0 x 10^9 cells/mL	0/3
Vibrio parahaemolyticus				ATCC 17802	5.5 x 10^8 cells/mL	0/3
Microaerophilic bacteria						
Campylobacter coli				ATCC 43479	1.8 x 10^8 cells/mL	0/3
Campylobacter jejuni				ATCC 33292	1.1 x 10^8 cells/mL	0/3

Helicobacter pylori	ATCC 43504	1.1 x 10^8 cells/mL	0/3
Anaerobic bacteria			
Clostridium bifermentans	ATCC 638	9.2 x 10^8 cells/mL	0/3
Bacteroides fragilis	ATCC 25285	1.1 x 10^9 cells/mL	0/3
Clostridium haemolyticum	ATCC 19398	1.1 x 10^9 cells/mL	1/3
Clostridium novyi	ATCC 19402	7.8 x 10^8 cells/mL	0/3
Clostridium orbiscindens	ATCC 49531	4.0 x 10^8 cells/mL	0/3
Clostridium perfringens	ATCC 13124	1.2 x 10^9 cells/mL	0/3
Clostridium scindens	ATCC 35704	8.3 x 10^8 cells/mL	0/3
Clostridium septicum	ATCC 12464	9.6 x 10^8 cells/mL	0/3
Clostridium sordellii	ATCC 9714	2.3 x 10^6 cells/mL	0/3
Clostridium sporogenes	ATCC 15579	1.2 x 10^9 cells/mL	0/3
Clostridium tetani	ATCC 19406	1.0 x 10^7 cells/mL	0/3
Klebsiella oxytoca	ATCC 33497	5.0 x 10^8 cells/mL	0/3
Lactobacillus acidophilus	ATCC 4356	8.9 x 10^8 cells/mL	0/3
Peptostreptococcus anaerobius	ATCC 27337	1.0 x 10^9 cells/mL	0/3
Porphyromonas asaccharolytica	ATCC 25260	6.9 x 10^7 cells/mL	0/3
Prevotella melaninogenica	ATCC 25845	9.4 x 10^8 cells/mL	0/3
Vibrio cholerae	ATCC 14035	1.3 x 10^9 cells/mL	0/3
Yersinia enterocolitica	ATCC 23715	9.0 x 10^8 cells/mL	0/3
Fungi			
Candida albicans	ATCC 10231	1.4 x 10^8 cells/mL	0/3
Viruses			
Adenovirus	Zeptometrix (Z) 0810084CF	1 x 10^7 TCID50/mL	0/3
Coxsachie virus	Z 0810074CF	1 x 10^7 TCID50/mL	0/3
Echovirus	Z 0810078CF	1 x 10^7 TCID50/mL	0/3
Rotavirus	Z 0810041CF	1 x 10^7 TCID50/mL	0/3
Astrovirus	Z 0810275CF	1 x 10^5 TCID50/mL	0/3
Cytomegalovirus	Z 0810003CF	1 x $10^{5.55}$ TCID50/mL	0/3
Norovirus Group I	Z 0810086CF	1 x $10^{5.39}$ TCID50/mL	0/3
Norovirus Group II	Z 0810087CF	1 x $10^{5.15}$ TCID50/mL	0/3
Enterovirus	Z 0810303CF	1 x $10^{6.34}$ TCID50/mL	0/3

* Abbreviations used: ATCC, American Type Culture Collection; CCUG, Culture Collection, University of Göteborg, Sweden; TCID, Tissue culture infective dose.

Supplemental Table 2. Potential interference by common endogenous and exogenous substances when tested with the Singulex Clarity C. diff toxins A/B assay.

Interfering substance	Interfering substance concentration	Sample type (TcdA/TcdB concentration)	TcdA/TcdB concentration (mean, pg/mL)	Detected Events prime signal (mean)	Interference
Mucin	0 mg/mL	High-negative	0.53	558.63	No
	3.5 mg/mL		0.63	570.39	
	0 mg/mL	Low-positive	6.03	1189.35	
	3.5 mg/mL		5.83	1213.77	
Human blood/Hemoglobin	0% v/v	High-negative	1.85	712.11	No
	50% v/v		1.94	722.62	
	0% v/v	Low-positive	10.50	1772.38	
	50% v/v		9.72	1675.46	
Barium sulfate	0% w/v	High-negative	5.22	1113.47	No
	20% w/v		5.54	1152.80	
	0% w/v	Low-positive	18.33	2794.10	
	20% w/v		16.81	2593.97	
Loperamide HCl	0 mg/mL	High-negative	4.95	1079.26	No
	0.1 mg/mL		4.34	1005.78	
	0 mg/mL	Low-positive	18.32	2792.48	
	0.1 mg/mL		16.99	2618.37	
Bismuth subsalicylate	0% v/v	High-negative	4.23	994.09	No
	10% v/v		4.93	1077.64	
	0% v/v	Low-positive	17.52	2687.00	
	10% v/v		15.88	2472.25	
Lipids	0 mg/mL	High-negative	5.06	1092.79	No
	20 mg/mL		3.53	907.74	
	0 mg/mL	Low-positive	17.63	2701.70	
	20 mg/mL		14.71	2318.96	
Metronidazole	0 mg/mL	High-negative	-0.59	429.56	No
	12.5 mg/mL		-0.91	391.97	
	0 mg/mL	Low-positive	1.95	723.45	
	12.5 mg/mL		0.67	574.99	
	0 mg/mL	High-negative	6.25	1322.83	
	6.25 mg/mL		3.01	937.45	
	0 mg/mL	Low-positive	15.63	2486.64	
	6.25 mg/mL		14.61	2356.08	

	0 mg/mL	High-negative	2.80	913.41	
	3.125 mg/mL		4.43	1100.50	
	0 mg/mL	Low-positive	20.09	3055.49	
	3.125 mg/mL		19.36	2962.83	
Acetaminophen	0 µmol/mL	High-negative	3.70	927.96	No
	1.34 µmol/mL		3.69	926.62	
	0 µmol/mL	Low-positive	20.27	3048.87	
	1.34 µmol/mL		17.96	2744.96	
Ibuprofen	0 µmol/mL	High-negative	5.28	1120.30	No
	2.43 µmol/mL		5.89	1197.15	
	0 µmol/mL	Low-positive	17.21	2647.40	
	2.43 µmol/mL		17.50	2684.99	
Vancomycin	0 mg/mL	High-negative	2.71	811.17	No
	12.5 mg/mL		2.54	792.19	
	0 mg/mL	Low-positive	15.32	2399.07	
	12.5 mg/mL		14.13	2243.61	
Stearic acid: Palmitic acid 1:1 ratio	0% w/v	High-negative	1.28	742.51	No
	40% w/v		3.02	938.47	
	0% w/v	Low-positive	10.24	1743.20	No
	40% w/v		9.85	1690.80	

Supplemental Table 3. Quantitative analyses of the stability of *C. difficile* samples was performed using the Singulex Clarity C. diff toxins A/B assay in a) different storage conditions and time points, and b) after three freeze-thaw cycles.

a)

	TcdA/TcdB concentration (pg/mL)								
	20–25 ºC		**2–8 ºC**		**-70ºC**				
	Day 0 (<4 Hours) Baseline	Day 0 (8 Hours)	Day 2	Week 1	Week 1	Week 2	Week 3	Month 3	Month 6
TcdA/TcdB-positive samples	1103.1	12403.8	8556.3	11328.2	14579.7	13558.6	13380.6	25696.3	14207.6
	4090.0	4111.7	7002.7	6278.8	6770.3	4792.4	6432.2	6463.2	6716.8
	15071.0	8560.1	4329.5	4348.5	7582.1	7402.2	10095.1	10932.7	7197.8
	3701.5	10469.2	3822.8	4981.9	4091.0	4447.7	4447.7	6036.6	4324.0
	19342.7	20330.2	25981.9	16145.2	30599.0	28072.2	16750.2	27298.4	23572.0
TcdA/TcdB-negative samples	-2.0	-1.5	6.5	-0.5	-3.0	-1.6	0.9	1.5	0.0
	-0.2	-0.5	0.2	0.2	-1.2	-2.1	1.4	7.1	0.5
	5.3	0.4	-2.4	-1.6	-1.5	-4.0	2.7	3.5	0.0
	-0.6	3.2	-1.6	-2.4	0.5	-0.5	0.2	2.0	0.0
	2.0	1.4	-2.0	-1.5	5.4	-2.9	0.1	0.7	0.1

b)

	TcdA/TcdB concentration (pg/mL)			
	Day 0 (<4 Hours) Baseline	First freeze-thaw	Second freeze-thaw	Third freeze-thaw
TcdA/TcdB-positive samples	1103.1	21112.4	12103.7	15288.9
	4090.0	6445.7	5591.3	6720.3
	15071.0	6189.4	5184.7	6009.7
	3701.5	4346.1	3751.7	3558.5
	19342.7	29124.9	20786.5	19587.2
TcdA/TcdB-negative samples	-2.0	-0.3	-1.2	-1.8
	-0.2	-0.7	-1.0	-1.1
	5.3	-2.5	-2.6	-2.7
	-0.6	-3.1	-2.2	-2.9
	2.0	-1.9	-0.9	-2.0

Supplemental Figure 1. *C. difficile* toxin concentration in stools containing 027 and non-027 strains.